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(54) Title: CLARIFIED AND COLD-MELT KONJAC GLUCOMANNAN (57) Abstract Clarified konjac glucomannan compositions substantially free of insoluble impurities, having a nitrogen content of no greater than about 0.60 wt %, and a turbidity potential as a 1.0 % aqueous sol of no greater than about 100 turbidity units as measured by the Formazin Turbidity Standard, as well as aqueous gels and sols, cold melt gels and spongy forms thereof. The resulting sols and gels may be used in foodstuffs, industrial biotechnical applications.		

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CLARIFIED AND COLD-MELT KONJAC GLUCOMANNAN

This invention relates to clarified konjac (that is, purified glucomannan derived from konjac) and methods for preparing the same. It includes clarified
5 konjac powders as well as sols and gels prepared therefrom. The clarified konjac glucomannan has enhanced purity and a low nitrogen content, and aqueous sols and gels thereof have low turbidity. This invention also relates to aspects of the clarified
10 konjac including a cold-melt product and to methods for making the above products as well as varying the clarified konjac viscosity.

Konjac (*Amorphophallus konjac*) is a plant, the tuber of which is the source of a well-known foodstuff
15 in China and Japan, namely konjac flour. This flour, which contains a variety of insoluble materials described below as well as a major amount of desirable water-soluble substances, comprises a highly viscous sol of glucomannan and soluble starches when
20 reconstituted in water. The principal soluble constituent is glucomannan, a polysaccharide comprised of D-glucose and D-mannose, which is useful as an ingredient in various foodstuffs, as well as in industrial applications such as films, oil drilling
25 fluids, and paints.

There are numerous impurities in crude (native, unclarified) konjac flour, principally insoluble starches, cellulose, and nitrogen-containing materials, including proteins, many of which impurities are
30 derived from "sacs" which encapsulate the konjac flour in the tuber. As a result, the sols and gels of crude konjac flour have a highly turbid, milky-white or cloudy appearance (due to water-swollen particulate impurities).

35 U.S. Patent 3,928,322 to Sugiyama et al. (and U.S.

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3,973,008, which is cumulative thereto) disclose a method for producing konjac mannan polysaccharide, i.e. glucomannan, which comprises the principal ingredient of konjac flour, from raw konjac flour by first
5 removing insoluble components from an aqueous konjac flour sol by filtration or other conventional means, thereafter dialyzing the sol and subjecting the resulting liquid to freeze-drying to obtain a turbid, cotton-like, low density fibrous product which is hard
10 to grind and poorly soluble in water.

Japanese Patent Disclosure 01-49657, filed March 1, 1989, discloses a konjac mannan product which has a nitrogenous component of not more than 0.2%. However, the method of achieving this reduced nitrogen content
15 is not disclosed but appears to be by simple dilution.

U.S. Patent 2,144,522 teaches a method for decolorizing and clarifying galactomannan gum sols such as locust bean gum which comprises contacting the gum sol with activated carbon in the presence of aluminum sulfate, the latter being added in amounts sufficient
20 to form a double Al-Na salt with sodium sulfate which is intrinsically present in the activated carbon itself.

U.S. Patent 3,346,556 discloses a method for
25 preventing the degradation of galactomannan gums such as locust bean gum resulting from heat or pH changes which comprises adding to aqueous gum sols polar organic oxygen-containing hydrophilic stabilizers such as alcohols, glycols, ketones or the like. Incidental
30 to this process there is disclosed in one example (Example 5) a means for clarifying locust bean gum by the conventional use of a filter aid such as diatomaceous earth.

Japanese Patent Disclosures 59-227,267 (Dec. 20,
35 1984), and 58-165,758 (Sept. 30, 1983) disclose methods

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for treating aqueous sols of crude konjac flour with certain salts at pH's of 10 or below to obtain an insoluble form of konjac, principally for use as insoluble food products.

- 5 Japanese Patent Disclosure 63-68054 (March 26, 1988), discloses a reversibly soluble konjac gel product, but not the removal of insolubles which remain present in the product.

- 10 Gels formed from combinations of glucomannan derived from crude konjac with other hydrocolloids, particularly polysaccharides such as carrageenan or xanthan gums, are already known in the art. See, for example U.S. Patent 4,427,704.

- 15 This invention provides dry clarified konjac glucomannan of low nitrogen content; aqueous sols and gels thereof; and methods for preparing each of the above products. The invention further provides: methods for varying the viscosity potential of the clarified konjac; co-processed hydrocolloids formed by
20 combining the clarified konjac with selected hydrocolloid gums; a cold-melt clarified konjac gel; and further method and product variations.

- The term "clarified" konjac, as used herein, refers to a konjac glucomannan which is substantially free of
25 insoluble impurities, which has a lower nitrogen content than unclarified konjac, and which exhibits a lower turbidity than unclarified konjac when in the form of an aqueous sol or gel. The term "crude" konjac, as used herein, refers to an unclarified or
30 native konjac flour in which the glucomannan is still contained in the sacs in which it occurs in nature, and various other impurities may be present.

- Generally, this invention encompasses clarified konjac characterized in that it comprises glucomannan
35 derived from konjac which is substantially free of

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insoluble impurities, and has a nitrogen content of 0 to 0.60 wt % accompanied by an aqueous sol turbidity potential of 20 to 70 Turbidity Units as well as a continuum of a nitrogen content of 0 to 0.25 %

5 accompanied by an aqueous sol turbidity potential of 70 to 100 Turbidity Units.

In a first group of embodiments this invention provides clarified konjac characterized in that it comprises glucomannan derived from konjac which is

10 substantially free of insoluble impurities; and [A] has a nitrogen content of from more than 0.25 up to about 0.60 wt % and an aqueous sol turbidity potential of from 20 to 70 turbidity units as measured at 1.0 w/v % concentration using the Formazin Turbidity Standard; as

15 well as the continuum of [B] a nitrogen content of 0.25 wt % or less, and an aqueous sol turbidity potential of 20 to 100 turbidity units as measured at 1.0 w/v % concentration using the Formazin Turbidity Standard, of which [B] is preferred. More preferably, the clarified

20 konjac is characterized by a nitrogen content of 0.175 wt % or less and an aqueous sol turbidity potential of 20 to 70 turbidity units. Most preferably, the clarified konjac is characterized by a nitrogen content of 0.15 wt % or less and an aqueous sol turbidity

25 potential of 20 to 60 turbidity units. This first group of embodiments also provides sols and gels of clarified konjac, cold-melt and spongy products, and methods for manufacturing the same.

In a second group of embodiments this invention

30 provides a clarified konjac characterized in that it comprises konjac-derived glucomannan which is substantially free of insoluble impurities, has a nitrogen content of about 0.60 wt % or less, and has an aqueous sol turbidity potential of less than 20

35 turbidity units as measured at 1.0 w/v % concentration

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using the Formazin Turbidity Standard; as well as a method for preparing the same. Preferably, the clarified konjac is characterized by a nitrogen content of no greater than about 0.25%. More preferably the clarified konjac is characterized by a nitrogen content of no greater than about 0.175%. This second group of embodiments also provides sols and gels of this clarified konjac and a method for its manufacture.

The clarified konjac of this invention also is characterized by an aqueous sol viscosity potential of about 50 to 25,000 cps at a 1 w/v % concentration as measured using a Brookfield Viscometer Model LVTDV-II at 25°C and 20 rpm, preferably a viscosity of about 1,000 to 25,000 cps.

Generally, the method for the production of clarified konjac of this invention is characterized by the consecutive steps of: [a] preparing an aqueous sol of crude konjac comprising insoluble impurities and glucomannan; [b] contacting the crude konjac sol with an extraction-effective amount of an agent capable of extracting the insoluble impurities; [c] precipitating and removing the insoluble impurities; [d] forming a glucomannan coagulate by treating the remaining aqueous sol with a coagulant present in an amount sufficient to coagulate substantially all glucomannan therein; and [e] removing and drying the glucomannan coagulate to recover the dry, clarified glucomannan.

In the first group of embodiments, the clarified konjac of this invention may be prepared by dispersing the konjac flour in water, and treating the resulting glucomannan dispersion with one or more reagents together or sequentially, to extract by aggregation, precipitation, or absorption of the impurities present. Such impurities are principally naturally-occurring in the konjac tuber and comprise nitrogenous materials

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such as proteins, insoluble fibers, and starches. Extraction is then followed by separating the impurities from the dispersion, coagulating the resulting glucomannan from the remaining dispersion by the addition of a water-miscible coagulant such as an alcohol, and drying and grinding the resulting coagulate to form the clarified konjac of this invention in powder form. These methods are particularly advantageous in that they can be completed significantly faster than known prior methods.

In the second group of embodiments, the method for production of the clarified konjac is characterized by the steps of:

- [a] preparing an aqueous sol of crude konjac comprising insoluble impurities and glucomannan;
- [b] contacting the crude konjac sol with an extraction salt selected from one or more of dicalcium phosphate, calcium phosphate, magnesium phosphate, and aluminum sulfate (preferably calcium sulfate and aluminum sulfate, more preferably aluminum sulfate) in an amount effective to extract the insoluble impurities by precipitation;
- [c] precipitating and removing the insoluble impurities;
- [d] forming a glucomannan coagulate by treating the remaining aqueous sol with isopropyl alcohol present in an amount sufficient to coagulate substantially all glucomannan therein; and
- [e] removing and drying the glucomannan coagulate to recover the clarified glucomannan.

Optionally, clarified konjac sols, alone or with other components, may be further converted into correspondingly pure gels by known methods, such as by addition of an alkali. The resulting gels may then be used in or as foodstuffs or in industrial compositions

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such as paints and other coatings.

The clarified konjac gels have the unusual property of liquifying within specific low temperature ranges. This is quite the reverse of the normal behavior of most hydrocolloid gels. Moreover, when cooled still further and then brought back to ambient temperature, the clarified konjac forms fibrous, porous, spongy, yet gel-like structures which, when compressed, rebound to their original form, and thus can serve as sponges to take up liquids and transport them to desired sites, such as to cells, seeds, calli, or plantlets placed within them.

The inventive methods afford additional advantages over unclarified (crude) konjac flour, namely improved odor, color, solubility, and grindability. Crude konjac has a known distinct odor, and a tan to dark brown color (as a dry powder). Furthermore, crude konjac particles are not uniform in size and cannot be ground at normal milling temperatures. Milling or other such grinding of crude konjac produces high temperatures which destroy its viscosity potential in much the same way as dry heat degradation, and which contribute to its dark color. By contrast, the clarified konjac of this invention is a white powder which forms a clear sol, is odor-free and can readily be ground to a uniform size. Additionally, clarified konjac is more uniform in glucomannan content, and thus avoids the wide, uncontrolled variations in viscosity or gel strength which occur with crude konjac.

Another desirable property of the clarified konjac powder of this invention is that, unlike crude konjac powder, clarified konjac hydrates rapidly in room temperature water with little effort, thereby facilitating the utilization of konjac in various recipes as well as the rapid preparation of sols of

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different viscosities.

Figure 1 compares the nitrogen and turbidity values of the clarified konjac of this invention obtained using various extraction agents, with those of prior art products, including crude konjac.

Figure 2 compares the UV absorbance properties of clarified konjac with crude konjac.

Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients, parameters, or reaction conditions used herein are to be understood as modified in all instances by the term "about".

The clarification of konjac to obtain a more purified glucomannan than is available from crude konjac affords several benefits, the most important of which is that clarified konjac sol is essentially clear, although a clarified konjac gel has some turbidity. Unexpectedly, when the clarified konjac sol is mixed with selected other hydrocolloid sols, and the mixture then gelled, there is a synergistic reaction which produces clear, thermally reversible gels. Such clear gels are particularly useful in forming desserts and for biotechnical applications where a clear gel is advantageous. Hydrocolloids particularly useful for synergistically combining with clarified konjac include clarified xanthan, locust bean gum, amylose and amylopectin starches, and carrageenan. Gel forming hydrocolloids such as agarose are merely additive and not synergistic. It is notable that crude xanthan and AMF (seaweed flour, sometimes sold as carrageenan) are not adequate for this purpose, because the combinations do not produced the desired clear gel. Clarified konjac gel alone is somewhat cloudy, although less cloudy than crude konjac gel.

Another important benefit of clarified konjac over

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crude konjac is that it is more stable as a dry powder.

For example, crude konjac stored for 4 weeks at above room temperature (50°C) loses 80% of its aqueous sol viscosity potential. By contrast, clarified konjac stored for the same time and at the same temperature loses only about 20% of its viscosity potential. It is believed that the increased storage stability is the result of the denaturing of enzymes present in the crude material, both by the initial heating of the sol and by the subsequent alcohol wash during the clarification process. It is a further benefit of clarification that clarified konjac is more easily rehydratable than crude konjac.

15 Crude Konjac Starting Material

Crude konjac flour starting material is a commercial product available from a number of sources. One source, and method for preparing konjac flour, is disclosed in Marine Colloids Bulletin K-1, "NUTRICOL® Konjac Flour" (1989) [product and bulletin of FMC Corporation, Marine Colloids Division, Philadelphia, Pennsylvania, 19103 U.S.A.]. Basically, the process involves slicing, drying and then wet- or dry-milling the *Amorphophallus tuber*, followed by pulverization of the resulting konjac to a powder ("flour") which is sifted and air classified. The resulting flour, as described in the above publication, consists of fine, oval, whitish granules containing "flour sacs", that is, the glucomannan is encapsulated in a protein/fiber coating. This flour, when hydrated for some time with agitation releases the encapsulated glucomannan to form a sol which is characterized principally by its high viscosity, even at 1% concentrations, substantial turbidity, and high nitrogen content. Viscosities in the range of 8,000 cps at a 1% by weight sol up to

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130,000 cps at 3% are typically obtained after a heat (85°C) and cool cycle, as measured on a Brookfield® RVT Viscometer, and an appropriate spindle, at 20 rpm and 25°C [the viscometer is a product of Brookfield

5 Engineering Laboratories, Inc. Stoughton, Mass., U.S.A.]. Conversion of Brookfield centipoise (cps) readings into viscosity functions are discussed by Mitschka, P. in Rheologica Acta, 21:207-209 (1982). As used herein, centipoise (cps) is equivalent to milli-
10 Pascals·second (mP·s).

The crude konjac turbidity may vary considerably, depending upon the concentration of the sol, but in the above viscosity range of from 8,000 cps up to 130,000 cps and concentrations of 1% to 3%, turbidities of 100
15 to 300 turbidity units are conventionally obtained at 0.5 wt. % concentration, based on the Formazin Turbidity Standard (FTS) - Method 180.1 in "Methods of Chemical Analysis of Water and Wastes" by EPA Environmental Monitoring and Support Lab; March, 1979.
20 At these turbidities the sol is generally very cloudy to milky in appearance.

The high nitrogen content of the initial crude konjac flour is essentially a function of the amount of impurities present, principally the tuber's naturally-
25 occurring protein and the sac fiber coating which encapsulates the glucomannan. The nitrogen content of the dry crude flour is typically in the range of 0.3 to 1.3 wt. % of nitrogen, although higher percentages are possible depending upon the variety of tuber used.

30

Product Description

As a measure of the significantly reduced amount of impurities present, the clarified products of this invention are characterized principally by their low
35 nitrogen content and low turbidity as an aqueous sol or

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gel. The corresponding viscosity of the product, in sol form, is also characteristically at a high level, and it is not adversely affected by the majority of agents that may be employed in the extraction process.

5 When prepared by the various methods of this invention, the clarified konjac is substantially free of insoluble impurities, having a nitrogen content and turbidity as low as possible. A 1.0% aqueous sol according to this invention should have no greater than
10 100 (preferably 70, more preferably 60), turbidity units, as measured by a MacBeth Coloreye Computer, model 1500, (Kollmorgen Corp., Newburgh, N.Y.), and a Formazin Standard; and has a nitrogen content, (based
15 sol), of generally no greater than 0.25 (preferably 0.175, more preferably 0.15) wt %. Within these ranges the clarified konjac sol is substantially transparent in appearance and may be used in a number of applications, particularly in clear foodstuffs and
20 biotechnical, or biomedical / diagnostic applications, where a clear, particle-free gel is essential, or where a highly viscous material is desired.

The product of this invention may further be described as having a very wide, non-critical range at
25 1.0 wt % aqueous sol of viscosities of from 50 to 25,000 centipoises, (as measured on a Brookfield Model LVTDV-II viscometer at 60 rpm and 25°C) depending upon how the product is prepared. In general, the clarified product inherently has high viscosities, i.e. from
30 1,000 to 25,000 cps, which are particularly useful for food formulations, but this viscosity can be reduced to as little as 50 cps by methods disclosed herein.

While the turbidity of flour and product samples is generally determined by using visible light,
35 ultraviolet (UV) light may also be employed to

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characterize the clarified product and gauge the effectiveness of clarification procedures. This may be achieved by preparing 0.5% sols of product, placing them in cuvettes and measuring their UV absorbance between 200 and 320 nanometers (nm). Impurities, including DNA and protein, absorb UV light in the 260-280 nm region and peaks in this area indicate their presence and relative amounts. As can be seen in Figure 2 and Table I, crude konjac samples contain a broad peak in this region and, overall, have a higher baseline of absorbance than clarified konjac samples, which lack the 260-280 peak. This is especially important for a biotechnology separation medium where the presence of DNA or protein might interfere with performance.

TABLE I

Konjac Sample	Absorbance			
	A320	A280	A260	A220
Crude	0.5140	0.8781	0.9634	2.6115
Clarified	0.0647	0.0967	0.1222	0.3118

Method Descriptions

The clarified konjac of this invention may be prepared by an aqueous extraction method comprising heating an aqueous sol of crude konjac flour containing insoluble impurities and contacting the heated sol with an extracting-effective amount of one or more extraction agents. The heating of the sol acts to break the natural sacs surrounding the glucomannan present in the crude konjac, and the extraction agent assists in removing protein impurities as well as the sacs themselves.

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The term "extracting" or "extraction", as used herein, means the separation of insoluble impurities from the konjac by aggregation, adsorption, precipitation or other means for rendering konjac flour substantially free of insoluble impurities.

Following the extraction, the sol is filtered to remove the insoluble impurities, and the filtrate coagulated with a water-miscible coagulating agent such as isopropyl alcohol to recover the glucomannan present. The coagulate is then dried and ground to particulate form, to produce a clarified konjac flour according to this invention.

The extraction step may be varied somewhat depending upon the nature of the extraction agent employed and the viscosity of the final product desired. For example, where the agent is a solid, it may be blended with the crude konjac flour starting material, optionally with a filter aid, and the dry mixture dispersed with agitation into a sufficient amount of water to obtain the desired concentration of the resulting clarified konjac glucomannan, 0.1 to 10 (preferably 0.5 to 3) wt % depending on the viscosity potential.

Alternatively, the extraction agent may be added to the water either before or after the aqueous dispersal of the flour, particularly if an acid is employed to adjust the viscosity of the resulting product. While this dispersion may be carried out in water at ambient temperatures, preferably the water should be heated to temperatures of from 70 to 100°C, (preferably 85 to 90°C) for 15 to 60 minutes or longer in order to speed up the process. Temperatures, times, mixing rates and concentration of reactants may be varied routinely by those skilled in the art in order to optimize these operating conditions.

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Thereafter, the sol is filtered to remove the insoluble impurities, with or without a filter aid present. Filters such as glass wool, paper, cloth and fibrous mats may be used for this purpose, although any
5 filter which will remove insoluble particles is generally satisfactory. Filter aids which may be employed include perlite and diatomaceous earth. The amount of filter aid is not critical, but is desirably employed in amounts of 1 to 5 times the weight of the
10 konjac flour. The filter cake is preferably then washed with hot water until no further clarified konjac glucomannan is recovered.

The filtrate is next treated with a water-miscible coagulating agent for the glucomannan, and the
15 coagulate recovered and dried. Useful coagulating agents include lower alcohols such as methanol, ethanol, or isopropyl alcohol or polar organic solvents such as acetone, methylethyl ketone, or mixtures thereof. The amount of coagulating agent is not
20 critical, but it should be added in amounts sufficient to recover the glucomannan from the sol generally in a weight ratio of 1-4:1, or a volume ratio of 2-3:1, coagulant:glucomannan. Alternatively, in place of coagulating agents the dry product may be recovered
25 directly from sol by such methods as freeze drying or spray drying.

The coagulate should be dried until it is capable of being ground to a fine powder. This may be achieved, for example in a forced hot air oven at
30 ambient temperatures, or even higher if viscosity reduction is desired. The resulting dry product is then ground to form particles of desired size, preferably capable of passing through a 100 mesh (149 micron) screen.

35 While the clarified, low-nitrogen product may be

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used in its dry, particulate form, for example in absorbent or texturizing applications, preferably it is used in sol form by redispersing the particles in water. The resulting clear sol may then be gelled in known manner and/or as described herein. The desired percent concentration of the dry composition in a sol or gel will depend largely on its intended use and its viscosity. Generally, 0.1 to 10 (preferably 0.5 to 3.0) wt %, based on the total weight of the sol may be employed, although these amounts are not critical. In addition, unless the process includes deliberate steps to reduce the viscosity of the product, it will normally maintain a very high viscosity despite the several treatment steps described herein.

As a further advantage of this invention, this clarified konjac sol normally develops a high viscosity, generally in the range of from 1,000 to 25,000 cps, at 1.0 w/v % (1 g/100 ml water) concentration and 25°C, as measured on a Brookfield Viscometer, Model LVT DV-II, with a suitable spindle at 12 rpm or on a model RVT at 20 rpm. Additionally, because of the rapid hydration properties of the clarified dry powder, it develops this viscosity rapidly. For example, when unclarified konjac flour is dispersed in water to form a 1 wt % sol, the hydration step normally takes about two hours at room temperature to form a sol of desired viscosity. By contrast, under substantially the same conditions, hydration of the product of this invention takes about 30 minutes to achieve the same viscosity. Optionally, when desired, the viscosity may be reduced to as low as about 50 cps at 1 w/v % and at 25°C.

The resulting sol may then readily be converted to a gel by known means, for example by addition of an alkali such as K_2CO_3 followed by heating. Unless the

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degree of polymerization has been deliberately modified during the processing, as described below, these gels generally possess a 1% gel strength at 85°C of from 100 to 310g/cm², when measured by a Marine Colloids Gel

5 Tester GT-2. (FMC Corporation, Marine Colloids Division, Philadelphia, Pennsylvania).

Extraction Agents and Means

In one embodiment, useful extracting agents are:

10 one or more salts selected from the group comprising dicalcium phosphate, calcium phosphate, magnesium phosphate, or aluminum sulfate (which is preferred), used together or sequentially.

Among suitable extraction agents are those useful

15 for changing the pH of the crude sol in order to refine the konjac, for example organic or inorganic acids such as HCl and bases such as NaOH. The amount of such agent employed should be extractive-effective, that is, sufficient to vary the pH from 1 to 8.5, preferably 3

20 to 8.5, within which ranges the benefits described in the product are obtained. At highly alkaline pH's the glucomannan, in addition to being extracted, may also begin to gel prematurely, while at highly acidic pH's the viscosity of the resulting product may be reduced.

25 However, if this latter viscosity reduction is desired, then beneficially both extraction and viscosity reduction can be achieved virtually simultaneously.

Aqueous extraction of the crude konjac may also be achieved by the use of chelating agents such as alkali

30 metal hexametaphosphates, ethylenediamine tetraacetic acid (EDTA), and nitrilotriacetic acid (NTA). The amount of chelating agent which should be used should be that which is chelating-effective, preferably 1 to 50 wt % based on the weight of the crude konjac.

35 Other useful extraction agents in the konjac

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clarification method are ion exchangers such as cation exchanging carboxymethyl cellulose (CMC), anion exchanging diethyl-[2-hydroxypropyl]aminoethyl cellulose (QAE), or diethylaminoethyl cellulose (DEAE),
5 desirably in extraction-effective amounts, preferably 5 to 15 wt % based on the weight of the crude konjac.

Soluble salts which may also be used as extraction agents include neutral salts such as sodium chloride; basic salts such as sodium acetate; or acidic salts
10 such as calcium chloride, or combinations thereof. Additionally, there may be used for this purpose a phosphate buffer, for example a 0.005 M buffer, pH 7.3, prepared by mixing monobasic sodium phosphate with dibasic sodium phosphate in suitable amounts. When
15 utilized, the soluble salt or buffer should be present in an extraction-effective amount, preferably 5 to 50 wt % based on the weight of the crude konjac.

In another embodiment, insoluble salts may be employed as extraction agents, for example dicalcium
20 phosphate, aluminum sulfate, calcium phosphate, magnesium phosphate, of which aluminum sulfate (alum) is preferred. If desired, these salts may be formed in situ during the extraction steps by known means. When utilized, the insoluble salts should be present in an
25 extraction-effective amount, preferably 1 to 25 (more preferably 5 to 15) wt % based on the weight of the crude konjac.

It also has been found that organic solvents including lower alcohols such as isopropyl alcohol may
30 be used for this purpose, as demonstrated in Example 15. When utilized, the organic solvents should be present in an extraction-effective amount.

Hot water alone (i.e. at 65 to 100°C) may be used as an extraction agent, although this is not
35 particularly satisfactory because turbidity may

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increase under some circumstances (see Ex. 47).

Certain Uses of Clarified Konjac

The amount of clarified konjac employed when
5 incorporated in foodstuffs or industrial compositions
will necessarily be varied, and can be determined
without undue experimentation by those skilled in the
art based on the known usage of crude konjac. For
example, in foodstuffs, amounts of 0.1 wt % may be used
10 in cake mix, while in industrial applications such as
films, oil drilling fluids, and paints, amounts ranging
from 1 to 2% and upward may be employed.

The use of the clarified glucomannan of this
invention in foodstuffs such as baked goods, dessert
15 gels, and meats, results in improved food properties.
For example, addition of the refined material to cake
dough results in improved texture, moistness, and rise
of the final product.

In its gel form the product is useful as a food or
20 food component, film former, and in various
biotechnical applications.

Viscosity Reduction

In accordance with a further aspect of this
25 invention the viscosity of the konjac sol, which is
normally high, may be reduced before, during, or after
the extraction step by treatment of the glucomannan
with a variety of reagents or other means to obtain
viscosities of whatever reduced values are desired.
30 Such sols of reduced viscosity are particularly useful
in biotechnology for preparing gels of high
concentration, and in cosmetics for texture control.
Moreover, if the viscosity is lowered before or during
the extraction step the subsequent filtration step is
35 naturally greatly facilitated, as is the general

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handling of the final product in sol form.

Means for reducing the viscosity of a 1.0 % w/v aqueous sol of the crude or clarified konjac (normally typically 14,500 cps) to 50 cps to 3,500 cps by depolymerization, for use in biochemical or pharmaceutical applications as well as for ease in handling, are known in the art and any suitable method may be used. Such means include: exposure to gamma radiation; exposure to radiation other than gamma such as actinic; acid hydrolysis, including "Smith Degradation" involving reduction of periodate-oxidized polysaccharide with borohydride, followed by mild hydrolysis with acid [see "Advances in Carbohydrate Chemistry and Biochemistry", Academic Press, New York, 1975, volume 31 page 203 et seq.]; alkaline hydrolysis; catalytic hydrolysis, for example by using iron EDTA (ethylenediaminetetraacetic acid) or NTA (nitrilotriacetic acid) with or without a transition metal addition; enzyme hydrolysis; mechanical shearing; thermal depolymerization such as by extended heating at 80 - 120°C in a dry or wet (aqueous sol) state; or other known means. Many known techniques and aspects of polymer degradation useful in this invention are described in "Elements of Polymer Degradation", by Reich and Stivala, McGraw Hill Book Co., New York, 1971.

Reduction of viscosity by irradiation can be achieved by contacting the crude or clarified konjac with gamma rays, such as generated from cobalt⁶⁰, at dosages ranging from 50 to 1200 Krad or above, in which case a direct correlation between dosage and viscosity is obtained, as shown in the examples below.

Alternatively, heat degradation of the crude or clarified glucomannan may be employed. For example, heating the glucomannan for a requisite number of

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hours, or even days, at temperatures of from 50 to 200°C, depending upon the reduced level of viscosity desired, will produce satisfactory results.

Among the chemical means, acid hydrolysis, or
5 contact with acid vapors of, for example, 5M HCl, during an acid extraction of crude konjac, with or without previous heating of the sol, produces a konjac sol of lowered viscosity which may be filtered more rapidly. It will be understood that this same method
10 also may be used to reduce the viscosity of the recovered product after it has been clarified. Reduction of viscosity with a base, on the other hand, where the pH remains above 12.5, yields unsatisfactory results in that the resulting dry product is either
15 discolored or insoluble in water, or both. Moreover, at a pH between 9 and 12.5, depending upon the base used, the sol will start to gel prematurely.

Cold Melt Gels / Sols

20 It has been found that the heat-set gel formed from a sol of the clarified konjac of this invention exhibits similar cold-melt properties to the crude konjac as used in the mentioned Japanese Patent Disclosures. A major difference is that, analogous to
25 the clarified konjac sols, the cold-melted clarified konjac gels form a clear liquid similar to a sol. Specifically, as a clarified konjac gel is cooled from room temperature, there is an almost linear softening of the gel and reduction in gel strength. At 10°C the
30 gel exhibits a visible softening and at 5°C it clearly becomes a liquid, whose nature has not been determined. The liquid state continues until about 0°C, below which point the clarified konjac gradually freezes into a solid (but not a gel). The cold liquid (at 5°C to 0°C)
35 will reform into a gel upon heating or warming. The

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re-formation of a cold-melt sol, upon re-chilling, has been observed on several occasions, and on one occasion a clarified konjac was reversed from gel to liquid to gel at least three times. When clarified konjac is cooled to freezing or slightly below and then brought back to room temperature, it forms a clear, water-insoluble, spongy, dimensionally stable mass. It is known that this phenomenon occurs with crude konjac, however the spongy mass formed with clarified konjac is noticeably lighter in color and contains none of the protein or other impurities found in crude konjac itself, and does not have the characteristic odor of crude konjac. Because of this, it is contemplated that the spongy mass prepared from clarified konjac is suitable for various medical applications such as implants and carriers for medications and for biotechnological applications requiring the absence of such contamination.

In order to ensure that the clarified konjac gel possesses this "cold-melt" property, it is important that it be formed under certain controlled conditions, primarily with respect to pH, as well as to the time the gel takes to form at any given temperature. Other factors which may also affect the ability of the gel to melt at low temperatures, include ion content and type. For example, it has been found that as the glucomannan concentration increases, the gel melts more slowly. However, the concentration of clarified glucomannan in the gel is not critical, and may vary from .01 to 10 (preferably 1 to 5) wt %.

In order to form a gel having cold-melt properties, the pH of the sol obtained from the clarified glucomannan first must be adjusted, desirably by heating it with an alkali at a temperature of from 65 t 130°C until the gel is formed. Th pH should

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desirably be 9.6 to 12.3, preferably 10 to 11.5, employing such bases as NH_4OH , NaOH , K_2CO_3 , or mixtures thereof, of which NH_4OH is preferred. It has been found, moreover, that gels formed at the lower pH values within this alkaline range subsequently melt to a sol more rapidly. In addition, the pH of already-formed gels which were prepared at high pH values, (see Example 11), can be lowered by treatment with a buffer solution, to a pH of 8-9 or lower without adversely affecting the cold-melt property of the gel. It has also been found that the cold-melt property is adversely affected by an extended gellation period, so that gel formation at elevated temperatures for short periods of time is preferable to lower temperatures for long periods.

Alternatively, it has been found that gels may be prepared at an acid pH (instead of alkaline pH) if the preparation is carried out under retort conditions, that is, at high temperatures while under pressure. For example, gels may be formed from clarified konjac sols at a pH of 6.7, a temperature of 130°C , and a pressure of 30 psi (about 2 atmospheres or 2.11 kgs/cm^2).

One convenient method for gelation is by adding NH_4OH to a 1% sol of clarified glucomannan until the desired pH is achieved, e.g. 11.2; heating the alkaline sol for about 5 to 60 minutes, depending upon the amount employed, (preferably 20 to 30 minutes at a temperatures of from 50 to 120°C , more preferably 80 to 90°C), until a gel forms; and thereafter cooling the gel in an ice bath, until it liquefies. The melted material can then be reformed to a gel by heating it until the gel starts to redevelop, generally starting at temperatures of 6°C and above.

In certain cases, notably when NH_4OH is used as the

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base, it has been found that as the gel melts, barely visible spherical particles may form in the liquid which, for purposes of any further clarification of the liquid, may be removed by filtration. As a theoretical explanation, it is believed that the outer surface of these coacervate-like particles contain water soluble starch which is present in the konjac flour.

Subject to those exceptions noted herein, the gels formed from the clarified konjac of this invention consistently exhibit cold-melt properties, that is, the gels liquefy when exposed to temperatures below 5°C, down to 0°C, at ambient pressure. If it is desired to keep the clarified konjac gel at low temperature without liquefying, this cold-melt property can be avoided by the admixture of non-cold-melt hydrocolloids, principally such gums as xanthan, carrageenans, and agaroids (especially agarose) or mixtures thereof. In some cases the clarified konjac will cogel with the hydrocolloid without the addition of alkali. Other hydrocolloids may require added alkali, heat, specific ions, or similar means to form the gel, as is known in the art.

It has also been found that at specific reduced temperatures, the presence of gums in the alkali-set gel results in the reversible transformation of a gel from a spongy texture to one which is a clear elastic.

In addition to the hydrocolloids, it has been found that certain ionic compounds at or above certain concentrations, for example salts such as NaCl, may also be used for the purpose of preventing cold melting of gels. In either case it will be seen that variant cold-melt properties can be achieved on a selective basis by the addition of these materials.

The amounts of hydrocolloid or ionic compounds necessary for preventing cold-melting of gels may be

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varied considerably. For example, the addition of 10% NaCl, i.e. ionic compound, by volume, will prevent cold melting. Alternatively, when a hydrocolloid is employed, the weight ratio of glucomannan to hydrocolloid in the gel may vary from about 10:1 to 1:10. For example, the addition of 1 part by weight carrageenan or xanthan to 3 parts of glucomannan, based on the weight of the konjac in the gel will likewise prevent cold melting. However, it will be understood that if it is desired to modify the properties of the gel in other respects as well, the amount of these additions employed can be increased accordingly.

The melted clarified konjac may be recovered in its liquid state and stored or handled that way, if desired, as long as it is maintained at temperatures generally below 50°C (at ambient pressure). In its cold, melted state the sol is notably stable at those temperatures. Alternatively, and more preferably, storage in the form of the gel at appropriate pH values until it is ready to be used, facilitates its handling.

The unique property of this cold-melt sol makes it highly useful in many ways, for example in biotechnology as an electrophoresis medium, or in medical technology as a drug delivery medium, e.g. by incorporation of a drug into the liquefied sol which could then be hardened by warming it for storage or administration purposes. Foodstuffs and beverages normally served cold could have their texture and consistency enhanced by making and/or storing a gel-containing food under cold conditions until ready to be served, e.g. frozen desserts or the like; or conversely, by adding the cold-melted sol to food in easily handled liquid form and then allowing it to set as a gel at room temperature.

In a further embodiment, the cold-melted sol may be

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used in cell encapsulation or to deliver drugs topically. That is to say, by incorporation of a water-soluble or suspended drug in the sol, as for example topical anaesthetics, antibiotics, antiseptics or the like, this sol, upon application to a cut or burn, dries to form a thin film that slowly releases effective amounts of the drug to the affected area.

EXAMPLES 1-21: EXTRACTION AGENTS

10 A series of experiments was carried out demonstrating the preparation of the clarified konjac product of this invention by means of the aqueous extraction of crude konjac flour with a variety of extraction agents. In each case (Examples 2-21) the
15 procedures of Example 1 were followed, except for the use of different extraction agents, as indicated. As also shown in Example 1, a sol of the dry, ground product was prepared after which a viscosity measurement was made.

20 Aqueous extractions, alone or incorporating various salts both soluble and insoluble, different pH's, chelating agents, ion exchangers, etc. were used. Time, temperature, konjac concentration and volumes were identical in these extractions. Filter aid usage
25 varied somewhat (0 to 100 g); where no filter aid was used the samples were filtered through a "cuno"-type cloth filter. This was done to speed the processing and was effective in removing the insoluble sacs; smaller microscopic particles remaining could readily
30 be removed with a filter aid, if desired. Coagulation (with isopropyl alcohol) washing and recovery were the same as in Example 1 in all examples except for routine modifications to suit specific cases.

As described in more detail below, in Examples 22-
35 42, alkali was then added to the corresponding sols of

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Examples 1-21 to form a gel, which was placed in crystallizing dishes and heated in a hot water bath. Gel strength was measured immediately and the gels placed directly in an ice bath to be observed for melting. The melted gel was then allowed to incubate at room temperature overnight and observed for its regelling ability. The results of each of these experiments are also summarized below in Table II.

10 EXAMPLE 1 - Hot Water

Six hundred ml of distilled water was heated to ~75 to 78°C in a hot water bath. Six grams of crude konjac was added and stirred for 60 minutes while maintaining this temperature range. A 1 liter pressure filter bomb was assembled using only a fitted piece of cuno filter cloth and then filled with boiling water which was then allowed to drain. The sample was poured into the filter bomb and 10 psi (0.7 kg/cm²) applied for 10 minutes. The pressure was gradually increased to 15, 20 25, 40, 45 psi (1.05, 1.75, 2.8, and 3.15 kg/cm²) and held at each level for 15 minutes. The total filtration time was 70 minutes and 430 ml filtrate was collected. The filtrate was coagulated in 2x volume of 99% isopropyl alcohol (IPA), based on the volume of the filtrate, and allowed to stand for 60 minutes. The coagulate was collected by vacuum filtration, on polyester cloth, squeezed dry, and transferred to 2x volume of 60% IPA for 30 minutes. The coagulate was again recovered, again treated with 99% IPA, and then dried at 55°C overnight (14 hours) in a forced hot-air oven. The sample weighed 3.57 g; (59.5% yield) and was ground through a 40 mesh screen (U.S. Standard Sieve Series). This material was used to prepare 200 ml of a 1 wt % aqueous sol by suspending 2 g in 200 ml distilled water. This was placed in a hot water bath

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(~80°C) and stirred with an overhead mixer for 45-60 minutes. The sample was poured into a 250 ml tall-form beaker and allowed to cool to 25°C. The viscosity was determined with a Brookfield Digital Viscometer (model
5 LVTDV-II) and found to be 18,400 cps (spindle #2, 0.3 rpm, 25°C, 91.7% of maximum).

EXAMPLE 2 (pH 2)

The following experiment illustrates the combined
10 viscosity reduction and extraction of crude konjac with acid at low pH. The effect was to reduce the viscosity before the extraction was completed.

The procedure outlined in Example 1 was repeated with the following changes. The water was adjusted to
15 pH 2 with 1.0M HCl before heating. Filtration was very quick, with 550 ml filtrate passing through the filter bomb in 8 minutes without the need to apply any pressure. The amount of time the coagulate sat after the initial coagulation was shortened to 45 minutes and
20 a final 15 minute hardening step in 99% IPA was employed. This process yielded 4.22 g (70.3%) of clarified glucomannan which had a 1% viscosity of 57.3 cps (Brookfield LVTDV-II spindle #1, 60 rpm, 25°C, 57% of maximum).

25

Examples 3 and 4 illustrate the aqueous extraction, as in Example 2, except that in Example 4 a base was used. It will be noted that while this procedure was fully effective at pH 7, at pH 10 the results were
30 poorer because of the partial gelling of the product at the higher pH values, which interfered with the filtration step.

EXAMPLE 3 (pH 7)

35 The procedure described in Example 2 was repeated

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using pH 7. The pH was controlled, as needed, with small amounts of 0.1N NaOH and 1.0N HCl. 250 ml of filtrate was collected in 95 minutes and then processed. The dried sample, 1.81 g or 30.1%, had a
5 viscosity of 14,200.

EXAMPLE 4 (pH 10)

In a like manner, as in Example 2, an aqueous extraction was carried out using pH 10 adjusted (1.0N
10 NaOH) water. Filtration was slow and only 150 ml of filtrate was collected (see table below for filtration times and pressures). The small amount of filtrate was observed to be partially gelled and was discarded.

15 Examples 5 and 6 illustrate the extraction process using two different chelating agents.

EXAMPLE 5 (Hexametaphosphate - HMP)

Sodium hexametaphosphate (3 g, 0.5% w/v) was added
20 to the hot water prior to the addition of the konjac. In this extraction, 50 g Celatom diatomite (Eagle-Picher; Cincinnati, Ohio) filter aid was mixed into the sample before filtration. After 109 minutes, 400 ml filtrate was collected and processed (see details
25 below). After drying, 3.62 g (60.3% yield) was ground and used to prepare a 1% sol. This material had a viscosity of 3,010 cps and a gel strength of 124 g/cm². It also "cold-melted" and regelled upon warming.

EXAMPLE 6 (Ethylenediamine Tetraacetic Acid-EDTA)

In a manner similar to Example 5, another 6 g crude konjac was extracted substituting 0.6 g (0.1% w/v) disodium EDTA for hexametaphosphate. Only 300 ml filtrate was collected after 120 minutes. This yielded
35 1.91 g or 31.9% after coagulation and drying. A 1% sol

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had a viscosity of 19,700 cps.

Examples 7-10 demonstrate the use of various soluble salts, or mixtures thereof, in the aqueous extraction of crude konjac, again using the general procedures of Example 1.

EXAMPLE 7 (Neutral Salt)

3 g NaCl was added prior to the addition of the konjac. The amount of filter aid was reduced to 25 g. 500 ml of filtrate was collected in 110 minutes and processed as above. This extraction produced 2.39 g (39.9% yield) of clarified konjac glucomannan having a 1% viscosity of 21,900 cps.

EXAMPLE 8 (Basic Salt)

3 g (0.5% w/v) sodium acetate was added to the water prior to the addition of the konjac. The filtrate (350 ml collected during 120 minutes) was processed, dried and then ground. The 3.674 g (61.2% yield) was used to prepare a 1% sol. The viscosity was measured at 4,660 cps.

EXAMPLES 9 AND 10 (Acidic Salt)

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was used in 2 other extractions 3.97 g; (0.5% w/v CaCl_2). In the first case (Example 9), 15 g filter aid Celatom diatomite (Eagle-Picher, Cincinnati, Ohio) was used but filtration was difficult. Only 50 ml filtrate was collected after 200 minutes and the experiment was subsequently abandoned.

A second attempt (Example 10) eliminated all filter aid. After 43 minutes, 525 ml filtrate was collected and processed yielding 4.06 g of dried product (67.7% yield). A 1% sol had a viscosity of 16,200 cps.

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EXAMPLE 11 (Phosphate Buffer)

A 0.005 M phosphate buffer, pH 7.3, was prepared by mixing 39 ml 0.2M monobasic sodium phosphate with 61 ml 0.2M dibasic sodium phosphate. An aliquot of this, 25 ml, was diluted to 1 liter giving a final concentration of 0.005M. Six grams of crude konjac was extracted in this solution as previously described. The filtrate, 250 ml obtained after 68 minutes, was processed and dried. The sample (1.957 g; 32.9% yield) had a 1% viscosity of 1,380 cps.

The following examples (12-14) demonstrate the aqueous extraction of crude konjac with ion exchangers and with a polar organic solvent, (Example 15). As noted in Examples 33-35 (below), the products of Examples 12-14 were found not to cold melt. This may be the result of ion binding and/or aggregation effects.

EXAMPLE 12 (Cation Exchanger-Carboxymethyl Cellulose)

(A.) To 600 ml distilled water, 0.6 g of water-soluble carboxymethyl cellulose (CMC) (10% w/w with konjac) was added before the addition of konjac. No filter aid was used and 500 ml filtrate was collected in 10 minutes at 5 psi (.35 kg/cm²). After processing and drying, 4.45 g or 74.1% was obtained. It had a 1% viscosity of 15,700 cps.

(B.) To 600 ml deionized water, 0.6 g of insoluble, microgranular CMC 32 (Whatman Labsales; Hillsboro, Oregon) was added before the konjac. No filter aid was used and 575 ml filtrate was collected in 32 minutes at pressures up to 20 psi (1.4 kg/cm²). After processing and drying, 3.90 g or 65.1% was obtained. It had a 1% viscosity of 15,900 cps and after gellation, did not cold melt.

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(C.) To 600 ml deionized water, 0.6 g of insolubl , fibrous CMC 23 (Whatman Labsales; Hillsboro, Oregon) was added before the konjac. No filter aid was used and 575 ml filtrate was collected in 23 minutes at pressures up to 10 psi ($.7 \text{ kg/cm}^2$). After processing and drying, 4.06 g or 67.6% was obtained. It had a 1% viscosity of 17,900 and also did not cold melt once a gel had been formed.

10 EXAMPLE 13 (Anion Exchanger Diethylaminoethyl Cellulose - DEAE)

0.6 g of DEAE cellulose was employed in an extraction. 500 ml of filtrate was collected in 35 minutes and was subsequently processed. The dried sample, 3.79 g or 63.1%, had a 1% viscosity of 16,100 cps.

EXAMPLE 14 (Anion Exchanger Diethyl-[2-hydroxypropyl]-aminoethyl Cellulose - QAE)

20 0.6 g QAE cellulose was added before the extraction. Filtration was not as good as that for DEAE; only 300 ml was collected in 74 minutes, which yielded 1.96 g (32.6%) after processing. This material had a 1% viscosity of 14,000 cps.

25

EXAMPLE 15 (20% Isopropyl Alcohol)

Six grams of the crude konjac was extracted in a mixture of 148 ml 99% isopropanol in 452 ml distilled water (20% w/w) as previously described. After 90 minutes of filtration, 275 ml of filtrate was collected and then processed. A total of only 1.25 g (20.9% yield) was recovered. This material had a viscosity of 11,800 cps.

35 In Examples 16-21 insoluble salts were used to

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adsorb impurities from the konjac in which the salts were formed in situ in Examples 18-21. It will be noted that when a filter aid was introduced by way of a modification to Example 18, as in Example 19, a clearer product was obtained. In a similar modification of Example 20, as in Example 21, but using a filter aid, much less filtrate was obtained.

EXAMPLE 16 (Dicalcium Phosphate)

Dicalcium phosphate was also used in an extraction by adding 0.6 g (10% w/w with konjac) to the water before the addition of the konjac. After the "cook", 15 g of filter aid was added and filtration was carried out for 10 minutes at 25 psi (1.75 kg/cm²) and then 40 minutes at 40 psi (2.8 kg/cm²). Only 50 ml of filtrate was collected during this time so the sample was removed from the filter bomb, pooled with the small amount of filtrate and an additional 85 g of filter aid mixed in. Filtration proceeded for 120 minutes during which time 250 ml of filtrate was collected and subsequently processed. This process yielded 1.65 g; (27.4% yield). This material had a 1% viscosity of 4,410 cps on a Brookfield Viscometer, Model LTVDV-II, No. 1 spindle.

25

EXAMPLE 17 (Aluminum Sulfate)

0.6 g aluminum sulfate was added prior to the konjac. After 35 minutes, 500 ml of filtrate was collected and then processed. The dried sample, 3.15 g or 52.6%, was ground and used to prepare a 1% sol and gel. The viscosity was determined to be 2,170 cps.

30

EXAMPLE 18 (Aluminum Sulfate - In Situ)

To 600 ml distilled water, 0.747 g monobasic sodium sulfate and 0.847 g aluminum chloride was added with

35

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stirring. No filter aid was used and 500 ml filtrate was collected after 11 minutes at 5 psi (0.35 kg/cm^2). This material was processed, dried and ground producing 4.20 g; (70% yield). This material had a 1% viscosity of 1,720 cps.

EXAMPLE 19 (Aluminum Sulfate - In Situ)

The above extraction was repeated with a few changes. 0.526 g of the aluminum chloride (0.291 g anhydrous AlCl_3) and 0.310 g monobasic sodium sulfate were used in this case. Additionally, 25 g of filter aid was added before filtration. Over 98 minutes, 450 ml of clear filtrate was collected and then processed. After drying, 3.65 g (60.9% yield) of material was ground and used to prepare a 1% sol. The sol, which was very clear, had a viscosity of 1150 cps.

EXAMPLE 20 (Dicalcium Phosphate - In Situ)

0.694 g calcium chloride and 0.567 g monobasic sodium phosphate was used in the extraction. Again, no filter aid was used and 340 ml filtrate was collected and processed producing 2.90 g; (48.3% yield). This sample had a 1% viscosity of 17,000 cps.

EXAMPLE 21 (Dicalcium Phosphate - In Situ)

The above extraction was repeated. The amount of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was reduced to 0.382 g (0.288 g anhydrous CaCl_2 or 2.6×10^{-3} mols) whereas the amount of NaH_2PO_4 dropped to 0.312 g (2.6×10^{-3} mols). Filter aid (25 g) was added before filtration, which proceeded slowly. Only 150 ml of filtrate was collected after 147 minutes. This extraction attempt was abandoned at this point.

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EXAMPLES 22-42: GEL AND COLD-MELT FORMATION

A series of runs was carried out wherein each of the clarified konjac products of Examples 1-21, respectively, was gelled and thereafter tested for gel strength and cold-meltability, as Examples 22-42. Results for these are all in below Table II.

It will be noted that of the recovered sols tested, only three of them, Examples 33-35, did not cold melt. In general, however, it will be seen that the vast majority of the gel products of this invention are cold-meltable.

EXAMPLE 22

To 200 ml of a 1% sol of the product of Example 1 was added, with stirring, 8 ml of 5M NH₄OH (i.e., 1 ml base/25 ml solution) to provide a pH of about 10.5. The sol was heated in a hot water bath for 60 minutes at a temperature of 85°C during which time a gel formed. The gel was immediately tested for gel strength, and then placed in an ice bath until a temperature of 4°C was obtained. The gel melted, as indicated in Table II, to form a substantially clear sol. When reheated, the gel reformed satisfactorily and was heat stable.

EXAMPLES 23-42

In accordance with the foregoing procedures of Example 22, but substituting as the starting materials the respective products of Examples 2-21 (where recovered) for the starting material of Example 22, there were obtained the corresponding gels. The gel strength and cold-melt properties of these gels is also reported in Table II.

TABLE II

<u>Example</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
Extraction Agent	dH ₂ O (a)	pH 2.0 (b)	pH 7.0 (b)	pH 10.0 (b)	HMP
Weight (w/w %)					3g (0.5%)
Filter Aid (c)	cloth only	cloth only	cloth only	cloth only	50g
Tot. Filter Time (min)	70	8	95	90	109
Tot. Filtrate Vol (ml)	430	550	250	150 (Abandoned)	400
Yield (g) / % Yield (d)	3.5 / (59.5)	4.220 / (70.3)	1.808 / (30.1)		3.621 / (60.3)
Viscosity (e) (cps)	18400	57.3	14200		3010
<u>Gel and Cold Melt Data</u>					
<u>Example</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>25</u>	<u>26</u>
Gel Strength (g/cm ²)	222	19	128	-	124
Cold-Melt	yes	yes	yes	-	yes

TABLE II (continued)

Example	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10 (f)</u>
Extraction Agent	EDTA	NaCl	NaOAc	CaCl ₂ ·2H ₂ O	CaCl ₂ ·2H ₂ O
Weight (w/w %)	0.6g (0.1%)	3g (0.5%)	3g (0.5%)	3.97g (0.5%)	3.97g (0.5%)
Filter Aid(c)	50g	25g	25g	15g	cloth only
Tot. Filter Time (min)	120	110	120	200	43
Tot. Filtrate Vol (ml)	300	500	350	50 (Abandoned)	525
Yield(g) / % Yield(d)	1.912/(31.9)	2.394/(39.9)	3.674/(61.2)	-	4.060/(67.7)
Viscosity(e) (cps)	19700	21900	4660	-	16200
Gel and Cold Melt Data					
Example	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>	<u>31</u>
Gel Strength g/cm ²)	171	310	204	-	194
Cold-Melt	yes	yes	yes	-	yes

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TABLE II (continued)

<u>Example</u>	<u>11</u>	<u>12-A</u>	<u>12-B</u>	<u>12-C</u>	<u>13</u>
Extraction Agent	5mM PO ₄ pH 7.3	CMC	CMC 32	CMC 23	DEAE Cellulose
Weight (w/w %)		0.6g (10%)	0.6g (10%)	(0.6g (10%))	0.6g (10%)
Filter Aid(c)	cloth only	cloth only	cloth only	cloth only	cloth only
Tot. Filter Time (min)	68	10	32	23	35
Tot. Filtrate Vol (ml)	250	500	575	575	500
Yield(g)/% Yield(d)	1.9/(32.6)	4.4/(74.1)	3.9/(65.1)	4.1/(67.6)	3.788/(63.1)
Viscosity(e) (cps)	1380	15700	15900	17900	16100
<u>Gel and Cold Melt Data</u>					
<u>Example</u>	<u>32</u>	<u>33-A</u>	<u>33-B</u>	<u>33-C</u>	<u>34</u>
Gel Strength (g/cm ²)	146	162	149	139	305
Cold-Melt	yes	no	no	no	no

TABLE II (continued)

<u>Example</u>	<u>14</u>	<u>15</u>	<u>16</u>	<u>17</u>
Extraction Agent	QAE Cellulose	2-propanol	CaH phosphate	(Al ₂ [SO ₄] ₃)
Weight (w/w %)	0.6g (10%)	0.6g (10%)	0.6g (0.1)	0.6g (0.1)
Filter Aid(c)	cloth only	cloth only	15 + 85	cloth only
Tot. Filter Time (min)	74	90	120	37
Tot. Filtrate Vol (ml)	300	275	250	400
Yield(g) / % yield(d)	1.974 / (32.9)	1.252 / (20.9)	1.646 / (27.4)	3.155 / (52.6)
Viscosity(e) (cps)	14000	11800	4410	2170
<u>Gel and Cold Melt Data</u>				
<u>Example</u>	<u>35</u>	<u>36</u>	<u>37</u>	<u>38</u>
Gel Strength (g/cm ²)	154	227	156	178
Cold-Melt	no	yes	yes	yes

TABLE II (continued)

<u>Example</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>
Extraction Agent	(Al ₂ [SO ₄] ₃)	(Al ₂ [SO ₄] ₃)	CaHphosphate	CaHphosphate
Weight (w/w %)	0.6g (10%)	0.6g (10%)	0.6g (10%)	0.6g (10%)
Filter Aid(c)	cloth only	25g	cloth only	25g
T t. Filter Time (min)	11	98	47	147
Tot. Filtrate Vol (ml)	500	450	340	150 (Abandoned)
Yield(g) / % Yield(d)	4.197/(70)	3.65g/(60.9)	2.902/(48.4)	-
Viscosity(e) (cps)	1720	1150	17000	-
<u>Gel and Cold Melt Data</u>				
<u>Example</u>	<u>39</u>	<u>40</u>	<u>41</u>	<u>42</u>
Gel Strength (g/cm ²)	225	104	283	-
Cold-Melt	yes	yes	yes	-

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FOOTNOTES FOR TABLE II

- a. *Distilled water*
- b. *pH adjusted and measured at room temperature*
- 5 c. *Filter aid: Celatom diatomite (Eagle-Picher; Cincinnati, Ohio)*
- d. *Dried coagulate, by weight, calculated as (wt. final product/wt. starting material) x 100*
- e. *1 wt % aqueous sol of dried coagulate*
- 10 f. *Same as Example 9 but without filter aid*
- g. *Measured in grams*

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EXAMPLES 43-47: ALUMINUM SULFATE AS EXTRACTION AGENT

An additional series of experiments (Examples 43-45) were carried out in accordance with the process of this invention to demonstrate the effect of aluminum sulfate as extraction agent on the clarity, nitrogen content and viscosity of konjac flour. The results of these examples are reported below in Table III, together with comparative examples 46 and 47.

In each of Examples 43-45, subject only to certain variations shown in the footnotes, 7 g of konjac flour (NUTRICOL® brand, FMC Corporation, Marine Colloids Division, Philadelphia, Pennsylvania), 22 g of the perlite filter aid (FW-40 from Chemrock Corp., Thomaston, Me.), aluminum sulfate (in varying amounts shown in the table), and 0.25 ml of 3M NaOH were added to 800 ml of H₂O (0.84 wt % konjac). The mixture was heated with agitation for 20 minutes at 90°C, filtered through a warm filter bomb under pressure ranging from 10 to 40 psi (.7 to 2.8 kg/cm²) and the filtrate precipitated into 3 volumes of 70% isopropyl alcohol to form a coagulate.

The coagulate was dried in a forced-draft oven overnight at 60°C to produce a hardened cake which was ground up through a No. 40 screen (U.S. Standard Sieve Series) (420 microns). The dry particulate product was then reconstituted as a 1 wt % aqueous sol. The viscosity and turbidity of this sol were then measured, the results of which are also reported in Table III.

In comparative Example 46, the viscosity and turbidity of a sample of commercial konjac flour dissolved in water to which no reagents had been added, was measured. In comparative Example 47, the dissolved konjac flour was processed in accordance with the method of this invention except that no aluminum sulfate or NaOH was added.

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TABLE III

EFFECT OF ALUMINUM SULFATE ON
VISCOSITY AND TURBIDITY OF KONJAC(a)

5		Example	Al Sulfate (gms)	Viscosity(b) (cp)	Turbidity(c) (T.U.)

		43	0.125	8,300	88
10		44	0.33	18,500	68
		45	0.66	6,200	54
		46(d)	None	11,800	174
		47(e)	None	6,790	189

15	a.	1 wt. % sol.			
	b.	Measured at 12 rpm on a Brookfield® Viscometer, Model LVTDV-II, (No. 4 spindle) at 1 wt % concentration and at 25°C.			
	c.	In Turbidity Units (T.U.) based on the Formazin Turbidity Standard as measured by a MacBeth Coloreye machine. These			
20		turbidity values were originally measured on a Fisher Spectrophotometer II, using a Fuller's Earth Standard. They			
		were then converted to the Formazin Standard by correlation studies which compared samples of the two standards, using			
		the Fisher unit. A final conversion to the MacBeth/Formazin			
25		standard of this table was then obtained via a correlation coefficient, as described below in Table VI, footnote (b).			
	d.	Unprocessed, untreated konjac flour.			
	e.	Processed with hot water (15 min. at 85°C), but not with			
30		aluminum sulfate or NaOH.			

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From the foregoing it will be seen, as shown in Example 44, that the viscosity of the clarified konjac may be maintained at very high levels, particularly for food use, by optimizing the amount of aluminum sulfate used. Also, the turbidity of the reconstituted, clarified konjac of this invention is significantly lower in Examples 43-45 of this invention as compared to the crude konjac sol of comparative Example 46. The processing (heating, etc.) of konjac with hot water in the absence of aluminum sulfate (Example 47) somewhat increased the turbidity of the reconstituted sol.

Of greater significance as shown in Example 44, at optimum concentrations of aluminum sulfate the viscosity of the reconstituted clarified konjac surprisingly, and desirably, increased when compared to that of Examples 46 and 47.

The following example illustrates a scaled-up version of the preceding aluminum sulfate clarification procedure.

EXAMPLE 48

To a 225 gallon (about 852 liter) stainless steel tank, 140 gallons (530 liters) cold water, 214 g aluminum sulfate (4.71%) and 162 ml 3M NaOH was added and heated with direct steam to 70°C. 10 lbs. (4536 g) konjac flour and 31 lbs. of FW 40 filter aid was mixed in. Total volume was 160 gallons (606 liters) which was 0.75% konjac w/v. The sample was heated to 85°C and held 15 minutes. The sample was then filtered in a preheated 18 inch stainless steel filter press with recycling occurring during the first 5 minutes. Total filtration time, including flushing the filter press with hot water, was 60 minutes. The filtrate was c agulat d in 300 gallons (1,136 liters) 85% isopropyl

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alcohol IPA. The coagulate was recovered by screening and by pumping it through bags which were subsequently squeezed in a small press. The coagulate was then washed/hardened in 75 gallons of 85% IPA for 2 hours with air agitation. The coagulate was recovered by screening and then squeezed by hand to remove excess liquid and subsequently dried at 55°C overnight. The sample, 6.4 lbs. (2.9 kg) or 64% yield, was ground through a 0.039 inch (2.4mm) screen. It had a nitrogen content of 0.15% and a 1% turbidity of 11 NTUs (Nephelometric Turbidity Units).

EXAMPLES 49-53: GEL AND COLD-MELT SOL FORMATION

In a further series of runs, and in accordance with the general procedures of Example 22, the product of Example 48 was tested for gelation and cold-meltability, using various bases and reaction conditions.

EXAMPLE 49

To 100 ml of a 1 wt % aqueous sol of the product of Example 48 was added, with stirring, 4 ml 5M NH₄OH to provide a pH of 10.08. The sol was heated in a boiling water bath for 20 minutes, during which the gel began to form after 7 minutes. The gel was then placed directly in an ice bath until it melted to form a substantially clear sol. When reheated, the gel reformed satisfactorily.

EXAMPLE 50 (Base variation)

The same procedure was followed as in Example 49, substituting sols of different bases for the ammonium hydroxide. Basic solutions used were 5M NaOH, 5M KOH, and 10% K₂CO₃ with adjustments made for minor concentration variations. The cold-melt phenomenon was

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observed with each; however, the formation of spherical bodies containing starch was only observed with the ammonium hydroxide cold-melt sol.

5 EXAMPLE 51 (Heating time variation)

Three 50 g samples of 1 wt % clarified konjac were prepared as in Example 49. To each was added 2 ml 5M NH₄OH with stirring. These were placed in a boiling water bath. Gelation was apparent after 7 minutes. At 10 20 minute intervals (20, 40 and 60 minutes) one beaker was removed. The gels were allowed to cool to room temperature and were then placed in an ice bath. All three gels cold-melted.

15 EXAMPLE 52 (pH variation)

A. Six 50-g aliquots of 1 wt % of aqueous clarified konjac were prepared in accordance with the procedures of Example 48. Using 1.0N NaOH and 0.1N HCl (to back titrate), each beaker was adjusted to one of 20 the following pH values: 8.5, 9.0, 9.5, 10.0, 10.5 and 11.0. These were all placed in a boiling water bath for 20 minutes. Those samples with initial pH values 10, 10.5 and 11 gelled and were removed after 20 minutes to cool at room temperature. Those samples at 25 lower pH did not gel after 1 hour in the water bath. The 3 gels were placed in an ice bath. The gel made at pH 10 melted fully and quickly. The gel made at pH 10.5 melted slowly and only partially. The last gel (pH 11.0) did not melt but softened considerably.

30 B. A series of 50-g aliquots of 1 wt % of aqueous clarified konjac was prepared as in Example 49. To the first four aliquots, all contained in beakers, 25, 50, 75 and 100 microliters, respectively, of 5M NH₄OH was added. The remaining aliquots received increments of 35 100 microliters (maximum 2.1 ml). The pH was checked

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by pH meter and visually, by universal indicator. The gels were then heat set for 20 minutes, cooled to room temperature, covered, and allowed to stand at room temperature overnight (16 hours). The beakers were all
 5 placed in an ice bath and monitored. The pH of those that melted was rechecked. Selected results are listed in the following table.

TABLE IV(a)

10

	<u>Volume NH₄OH (μl)</u>	<u>Initial pH</u>	<u>Gelled</u>	<u>Melted</u>	<u>Final pH</u>
	25	9.5	no	---	9.2
	50	9.8	no	---	8.8
	75	9.8	weak	yes	8.9
15	100	10.0	yes	yes	9.0
	500	10.4	"	"	9.8
	1000	10.8	"	"	10.1
	1500	11.3	"	"	na
	2000	11.4	"	partial	na

20

A similar series was run using 5M NaOH instead of NH₄OH. The results were as follows:

TABLE IV (b)

25

	<u>Volume NaOH (μl)</u>	<u>Initial pH</u>	<u>Gelled</u>	<u>Melted</u>	<u>Final pH</u>
	50	9.2	no		7.3
	175	10.4	"		8.2
30	200	10.6	weak	yes	8.5
	225	10.8	yes	"	8.6
	250	11.0	"	"	8.7
	300	11.2	"	"	8.8

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The following example illustrates the preparation of a gel at retort conditions and at a low pH.

EXAMPLE 53

5 For this example, 800 mls of a 2% sol of clarified konjac from Example 48 was prepared by dispersing and dissolving it in a pH 6.6 phosphate buffer. This material was used to fill an aluminum can to capacity which was subsequently sealed. The can was placed in a
10 pressure cooker and heated at 130°C at 30 psi (2.1 kg/cm²) for 60 minutes. After cooling, the can was opened. A soft gel was revealed. Several pieces were removed, placed in a separate small beaker and then iced. The gel melted fully and when heated in a hot
15 water bath (~90°C) for 25 minutes, a much firmer gel reformed.

EXAMPLE 54 (Gel and cold-melt sol stabilities)

Eight 100-g 1 wt % clarified konjac sols were
20 prepared in accordance with the procedures of Example 48. The following volume of 5M NH₄OH was added, in duplicate, to the samples (resulting pH value is in parentheses): 1 ml (pH 10.39), 2 ml (pH 10.58), 3 ml (pH 10.78) and 4 ml (pH 10.90). All eight samples were
25 heat set for 20 minutes in a boiling water bath. Four of these gels, one at each level, were covered with plastic wrap and allowed to stand at room temperature for 10 days. The other four gels were placed in an ice bath after cooling. Only the gels formed at pH 10.39
30 and 10.58 melted. The other two gels at pH 10.78 and 10.90, softened but did not melt. All four were covered with plastic and stored in a 9°C refrigerator at 9°C.

The samples stored in the refrigerator were

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examined after 8 days. The two lower pH aliquots were still in molten form, while the two higher pH samples were unchanged (soft gels). Small samples of the two cold melts were placed in a test tube and placed in a boiling water bath for 10 minutes. Both formed gels, but these gels did not remelt.

The gels stored at room temperature were placed in an ice bath to check for meltability. The two lower pH gels melted completely. The remaining gels, at pH values of 10.78 and 10.90, melted substantially but not fully.

The following examples illustrate additional methods for reducing the viscosity of the clarified konjac of this invention by means of irradiation.

EXAMPLES 55-63 (Viscosity Reduction by Irradiation)

Six 50 g aliquots of clarified konjac obtained by the process of Example 48, and a 100 g portion of an alcohol-washed crude konjac sample were irradiated by gamma rays (cobalt 60).

Sols (200 ml, 1% w/v) of each sample, as well as samples of the original nondegraded materials, were prepared by heating the sample in a water bath and stirring with an overhead mixer for 60 minutes. The samples were poured into 250 ml tall-form beakers and allowed to cool to room temperature. The viscosities were determined with a Brookfield digital viscometer as described above. An aliquot (50 ml) of each sample was mixed with 2 ml of 5M NH₄OH and placed in a boiling water bath for 20 minutes to check for gelling ability. Following gelation, the gels were placed on ice to check for cold-meltability. The results of each of these tests are shown below in Table V.

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TABLE V

	Example	Konjac	Irrad. Level	Viscosity	Gel	
			(Krad)	(cps)	Formed	Melted
5	55	Clarified	0	3360	yes	yes
	56	Clarified	50	1800	yes	yes
	57	Clarified	100	1230	yes	yes
	58	Clarified	200	641	yes	yes
	59	Clarified	300	357	yes	yes
10	60	Clarified	600	129	yes	yes
	61	Clarified	900	58	weak	yes
	62	Crude	0	11200	yes	yes
	63	Crude	300	1630	yes	yes

15

EXAMPLES 64-82 (Nitrogen and Turbidity Content
of Clarified Konjac

20 Selected products obtained from previous examples were measured to determine their nitrogen content and turbidity level. (Ex. 64-74). These results were compared with the nitrogen content and turbidity level of both crude konjac flour, (Ex. 76 and 78-82) and the products of the process described in U.S. Patent

25 3,928,322, (Ex. 75) as well as those of the product of Ogasawara et al., described in "Electrophoresis on Konjac Mannan Gel", Seibutsu Butsuri, 31, pp. 155-158 (1987), (Ex. 77) which represents a slight modification of the U.S. 3,928,322 process. The results of all of

30 these tests are set forth below in Table VI, and in Figure 1, wherein all nitrogen values are based on the dry weight of the product.

In Table VI, as described in footnote (b), certain of the turbidity values were first obtained on a Fisher

35 Spectrophotometer, Model II (Fisher Scientific,

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Pittsburgh, Pa.), using a Formazin Standard and then converted to MacBeth Coloreye values. This conversion was carried out through a correlation study, as follows: 11 Formazin standards, with turbidity values ranging from 5 to 400 NTU's, were prepared and measured (% transmittance) on both the MacBeth and Fisher units. Additionally, 5 konjac samples (3 crude and 2 clarified) were prepared at concentrations of 1%, 0.5%, 0.25% and 0.125% and also measured on both units. The data (% transmittance) from these measurements were plotted against their turbidity values, as determined on each machine, and a correlation coefficient determined.

- 15 The process of U.S. Patent 3,928,322 - Sugiyama (Example 75) was carried out as follows:
1. 2.5 g konjac flour (89-9607) was suspended in 500 ml (0.5% w/v) tap water and heated at ~55-60°C for 2 hours.
 - 20 2. The sol was passed through a 115 mesh (125 micron) and then a 270 mesh (53 micron) metal screen to remove gross insolubles.
 3. The sol would not filter through a medium porosity glass filter (Pyrex 150 ml, ASTM 10-15) or a 0.2 micron filter so instead was heated to 90°C and twice passed through a 14 inch - 1 inch diameter (35.6 cm - 3.54 in diameter) bed of tightly packed glass wool. The filtrate, 300 ml, was very clear and appeared to be particle-free.
 - 25 4. The filtrate was placed in a piece of dialysis tubing (Spectra/Por, 47.7 mm x 75 mm, molecular weight cut off of 12 -> 14,000 daltons). The sample was dialyzed against 4 liters of tap water for 48 hours (the water was changed after 24 hours).
 - 30 hours).
 - 35

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5. The sample was then poured into 2 large crystallizing dishes and frozen.
6. Each aliquot was lyophilized at 0.6 Torr with a shelf temperature of 100°F (37.8°C) for 12 hours.
- 5 7. The dried sample was very white and quite fluffy. The yield was 1.137 or 45.5%. Due to excessive static, the sample could not be ground and was wetted with a small amount of 20% isopropyl alcohol and then dried at 55°C for 3 hours. The sample was
- 10 then ground through a 40 mesh screen.
8. The sample had a nitrogen content of 0.07% and a 1.0% turbidity of 128 Turbidity Units.
9. The process took a total of 68 hours to run.

15

The Ogasawara process (Example 77) was carried out as follows:

1. 10 g crude konjac was suspended in 100 ml 50% ethanol and stirred for 1 week.
- 20 2. This material was centrifuged and the pellets were transferred to 100 ml 80% ethanol for 3 days with stirring.
3. This was again centrifuged (4000 rpm, 10 minutes) and the pellets transferred to 100 ml 100% (absolute) ethanol for 1 hour.
- 25 4. The sample was recovered on #54 Whatman filter paper by vacuum filtration, and dried in a 60°C oven for 6 hours.
5. 8.992 g of material was recovered and was used to
- 30 prepare a 5% sol in 178 ml. This was too viscous to treat, and was diluted 10 fold to 1780 ml (0.5% w/v) and allowed to sit overnight at room temperature.
- 35 6. This material was centrifuged for 75 minutes at 9500 rpm.

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7. The supernatant (1700 ml) was dialyzed in 10 volumes of distilled water for 3 days at room temperature.
8. The sample was removed from the dialysis tubing and centrifuged at 7500 rpm for 10 minutes.
9. Half of the supernatant was coagulated while the other half was placed in dialysis tubing and covered with polyethylene glycol (PEG 20) to reduce the volume from 850 ml to 450 ml.
10. This material was frozen at -75°C for 45 minutes and then lyophilized at 0.1 Torr and 100°F (37.8°C) for 3 days.
11. 2.19 g of the lyophilized material was recovered and was very white and fluffy in appearance.
12. All samples were vacuum dried to remove any moisture before testing. The lyophilized material foamed excessively when the sol was prepared for turbidity measurements.
13. This process took a total of ~384 hours to run.

20

TABLE VI

	<u>Example</u>	<u>Corresp. Fig.</u>	<u>Extraction Agent</u>	<u>From Example</u>	<u>% Nitrogen^(c)</u>	<u>1.0% MacBeth^(d) Turbidity</u>
25	64	1	Soluble CMC	12	0.09	4 ^(b)
	65	2	Alum, pilot plant	48	0.07	7
	66	3	pH 2	2	0.08	10
	67	4	Alum, pilot plant	48	0.07	18
	68	5	Alum, pilot plant	48	0.15	11
30	69	6	Alum, pilot plant	48	0.13	14
	70	7	Sodium Acetate	8	0.09	45
	71	8	Hot water	1	0.07	45 ^(b)
	72	9	HMP	5	0.06	45 ^(b)
	73	10	Insoluble CMC 23	12	0.03	50
35	74	11	Insoluble CMC 32	12	0.04	50
	75	12	Sugiyama Patent	(above)	0.07	128 ^(b)
	76	13	Crude/Hydro washed		0.11	177

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	<u>Example</u>	<u>Corresp.</u> <u>Fig.</u>	<u>Extraction Agent</u>	<u>From</u> <u>Example</u>	<u>% Nitrogen</u> ^(c)	<u>1.0% MacBeth</u> ^(d) <u>Turbidity</u>
5	77	14	Ogasawara Publ.	(above)	0.31	80(b)
	78	15	Crude		0.32	92(a)
	79	16	Crude		0.30	101(e)
	80	17	Crude		0.29	120(e)
	81	18	Crude		0.41	155(a)
10	82	19	Crude		0.59	142(e)

-
- a. sample centrifuged at 4000 rpm for 5 minutes before analysis
- b. the values originally obtained on a Fisher Spectrophotometer,
15 then converted into MacBeth equivalents as determined by a
regression line obtained by plotting values obtained from a
correlation study of identical samples measured on both
instruments.
- c. based on the dry weight of the product.
- d. as measured on a MacBeth Coloreye Computer (Series 1500),
20 using a Formazin Standard.
- e. 0.5% solution turbidity.

From the foregoing results it will be seen that
whereas the nitrogen and turbidity values of the
25 products of this invention (Examples 64-75) were both
low, the corresponding values of the crude konjac, as
well as one or both of the Sugiyama and Ogasawara
products, were significantly higher by comparison.

30 The following example illustrates the inhibiting
effect of hydrocolloids on the cold-melt properties of
the clarified gels of this invention.

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EXAMPLE 83 (Added Hydrocolloids Gums)

Xanthan: 100 g of a hot 1% clarified konjac sol was mixed with 33 g of a 1% w/v sol of xanthan (Keltrol T, Kelco Co., San Diego, Ca.). The mixture, which
5 began gelling almost immediately, was heated in a hot water bath to melt the gel. Once melted, two 50 g aliquots were poured into beakers. Two ml of 5M NH₄OH was stirred into each hot liquid sample. One was placed in a boiling water bath for 20 minutes while the
10 other was allowed to cool to room temperature. Both samples formed gels although they differed in appearance and texture. The heat set gel was opaque and somewhat spongy while the second aliquot (not heat set) was clear and very elastic. The heat set gel,
15 when placed in an ice bath, became clear and elastic but did not liquefy. When this transformed gel was reheated, it took on its original properties, that is, opaque and spongy. When placed in an ice bath, it again reverted to the clear elastic gel.

20 Carrageenan: 33 g of a 1% w/v CIC carrageenan sol (sodium, reduced-viscosity kappa-form, a product of FMC Corporation, Marine Colloids Division, Philadelphia, Pennsylvania) was mixed with 100 g of a 1% clarified konjac sol. Five ml of 5M NH₄OH was added with
25 stirring and the sample was heat set for 20 minutes. A soft opaque gel formed which, when placed in an ice bath, was transformed into a clear very elastic gel, but did not liquefy.

Agarose: 5 x 67 g samples of a 1% 3:1 clarified
30 glucomannan/agarose sol were prepared by mixing 50 g of a 1% konjac sol with 16.7 g agarose sol (SeaKem® LE agarose, FMC Corporation, Marine Colloids Division, Bioproducts Group, Philadelphia, Pennsylvania). Two ml of 5M NH₄OH were added to four of the aliquots and two
35 of these were heat set in a boiling water bath for 20 minutes.

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All five samples formed gels. Those gels formed with base and heat were opaque and very soft. The gels which were not heat set (two with base, one without) were clear and tough. When a heat set gel was placed
5 in an ice bath, it did not melt but was transformed into a clear tough gel, analogous to the non heat-set samples.

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CLAIMS:

1. Clarified konjac characterized in that it comprises glucomannan derived from konjac which is substantially free of insoluble impurities; and

5 [A] has a nitrogen content of from more than 0.25 up to about 0.60 wt % and an aqueous sol turbidity potential of from 20 to 70 turbidity units as measured at 1.0 w/v % concentration using the Formazin Turbidity Standard; as well as in a continuum

10 [B] a nitrogen content of 0.25 wt % or less, and an aqueous sol turbidity potential of 20 to 100 turbidity units as measured at 1.0 w/v % concentration using the Formazin Turbidity Standard.

2. The clarified konjac of claim 1 characterized
15 by a nitrogen content of 0.25 wt % or less, and an aqueous sol turbidity potential of 20 to 100 turbidity units.

3. The clarified konjac of claim 1 characterized
20 by a nitrogen content of 0.175 wt % or less and an aqueous sol turbidity potential of 20 to 70 turbidity units.

4. The clarified konjac of claim 1 characterized
25 by a nitrogen content of 0.15 wt % or less and an aqueous sol turbidity potential of 20 to 60 turbidity units.

5. The clarified konjac of claim 1 characterized
by an aqueous sol viscosity potential of 50 to 25,000
cps at a 1 w/v % concentration as measured using a
Brookfield Viscometer Model LVTDV-II at 25°C and 12
30 rpm.

6. The clarified konjac of claim 5 characterized
by a viscosity of 1,000 to 25,000 cps.

7. The clarified konjac of claim 1, 2, 3, 4, 5,
and 6, characterized in that it comprises an aqueous
35 s l.

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8. The clarified konjac of claim 1, 2, 3, 4, 5, and 6, characterized in that it comprises an aqueous gel.

5 9. The clarified konjac of claim 8, characterized in that it comprises a mixture with at least one additional hydrocolloid before said gel is formed.

10 10. The clarified konjac gel of claim 9, characterized in that the additional hydrocolloid is selected from among carrageenan, xanthan, and agarose.

11. The clarified konjac gel of claim 10 characterized in that the weight ratio of clarified konjac to hydrocolloid in the gel mixture is about .1-10:1.

12. A clarified konjac comprising glucomannan
15 derived from konjac which is substantially free of insoluble impurities; has a nitrogen content of 0.60 wt % or less; and has an aqueous sol turbidity potential of less than 20 turbidity units as measured at 1.0 w/v % concentration using the Formazin Turbidity Standard;
20 characterized in that it comprises a mixture with at least one additional hydrocolloid before said gel is formed.

13. The clarified konjac gel of claim 12, characterized in that the additional hydrocolloid is
25 selected from among carrageenan, xanthan, and agarose.

14. The clarified konjac gel of claim 12, characterized in that the weight ratio of clarified konjac to hydrocolloid in the gel mixture is about .1-10:1.

30 15. The clarified konjac of claim 1 characterized in that it is in the form of a clear, water-insoluble, spongy, dimensionally stable mass.

16. A clarified konjac comprising glucomannan derived from konjac which is substantially free of
35 insoluble impurities; has a nitrogen content of ab ut

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0.60 wt % or less; and has an aqueous sol turbidity potential of less than 20 turbidity units as measured at 1.0 w/v % concentration using the Formazin Turbidity Standard; characterized in that it is in the form of a clear, water-insoluble, spongy, dimensionally stable mass.

17. The clarified konjac of claim 1 characterized in that it is an aqueous cold-melt gel at temperatures above about 5°C which reversibly liquifies to a clear sol at temperatures between 5°C to 0°C.

18. A clarified konjac comprising glucomannan derived from konjac which is substantially free of insoluble impurities; has a nitrogen content of 0.60 wt % or less; and has an aqueous sol turbidity potential of less than 20 turbidity units as measured at 1.0 w/v % concentration using the Formazin Turbidity Standard; characterized in that it is an aqueous cold-melt gel at temperatures above 5°C which reversibly liquifies to a clear sol at temperatures between 5°C to 0°C.

19. A method for the production of clarified konjac characterized by the consecutive steps of:

[a] preparing an aqueous sol of crude konjac comprising insoluble impurities and glucomannan;

[b] contacting the crude konjac sol with an extraction-effective amount of an agent capable of extracting the insoluble impurities;

[c] precipitating and removing the insoluble impurities;

[d] forming a glucomannan coagulate by treating the remaining aqueous sol with a coagulant present in an amount sufficient to coagulate substantially all glucomannan therein; and

[e] removing and drying the glucomannan coagulate to recover the dry, clarified glucomannan.

20. The method of claim 19 characterized by

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selecting the extraction agent from among: chelating agents; soluble salts; insoluble salts; ion-exchangers; organic solvents; hot water; or means for adjusting the pH of the sol.

5 21. The method of claim 19 characterized in that the extraction agent is an insoluble salt.

22. The method of claim 19 characterized in that the coagulant is isopropyl alcohol.

10 23. The method of claim 19 characterized by treating the aqueous crude konjac flour with a viscosity-reducing agent prior to extraction.

24. The method of claim 19 characterized by treating the aqueous sol remaining after extraction with a viscosity-reducing agent prior to treatment with
15 the coagulant.

25. The method of claim 19 characterized by treating dry clarified glucomannan or an aqueous sol thereof before, during or after clarification with a viscosity-reducing agent.

20 26. The method of claim 19 characterized in that the viscosity-reducing agent is an acid which is simultaneously used as an extraction agent.

27. The method of claim 19 characterized in that the viscosity-reducing agent is gamma ray irradiation.

25 28. A method for the production of the spongy, dimensionally-stable mass of claim 15 characterized in that clarified konjac aqueous sol is cooled to freezing temperature or slightly below and then brought back to room temperature.

30 29. A method for the production of the spongy, dimensionally-stable mass of claim 16 characterized in that clarified konjac aqueous sol is cooled to freezing temperature or slightly below and then brought back to room temperature.

35 30. A method for the production of the cold-melt

- 60 -

gel of claim 17 characterized in that the pH of a clarified konjac aqueous sol is adjusted to between about 9.6 and 12.3 before gel formation.

31. The method of claim 30 characterized in that
5 the pH is adjusted to between 10.0 and 11.5.

32. The method of claim 30 characterized in that said gel formation is effected while heating for 5 to 60 minutes at a temperature of 50 to 120°C.

33. The method of claim 31 characterized in that
10 said gel formation is effected while heating for 20 to 30 minutes at a temperature of 80 to 90°C.

34. A method for the production of the cold-melt gel of claim 18 characterized in that the pH of a clarified konjac aqueous sol is adjusted to between 9.6
15 and 12.3 before gel formation.

35. The method of claim 34 characterized in that the pH is adjusted to between 10.0 and 11.5.

36. The method of claim 34 characterized in that said gel formation is effected while heating for 5 to
20 60 minutes at a temperature of 50 to 120°C.

37. The method of claim 35 characterized in that said gel formation is effected while heating for 20 to 30 minutes at a temperature of 80 to 90°C.

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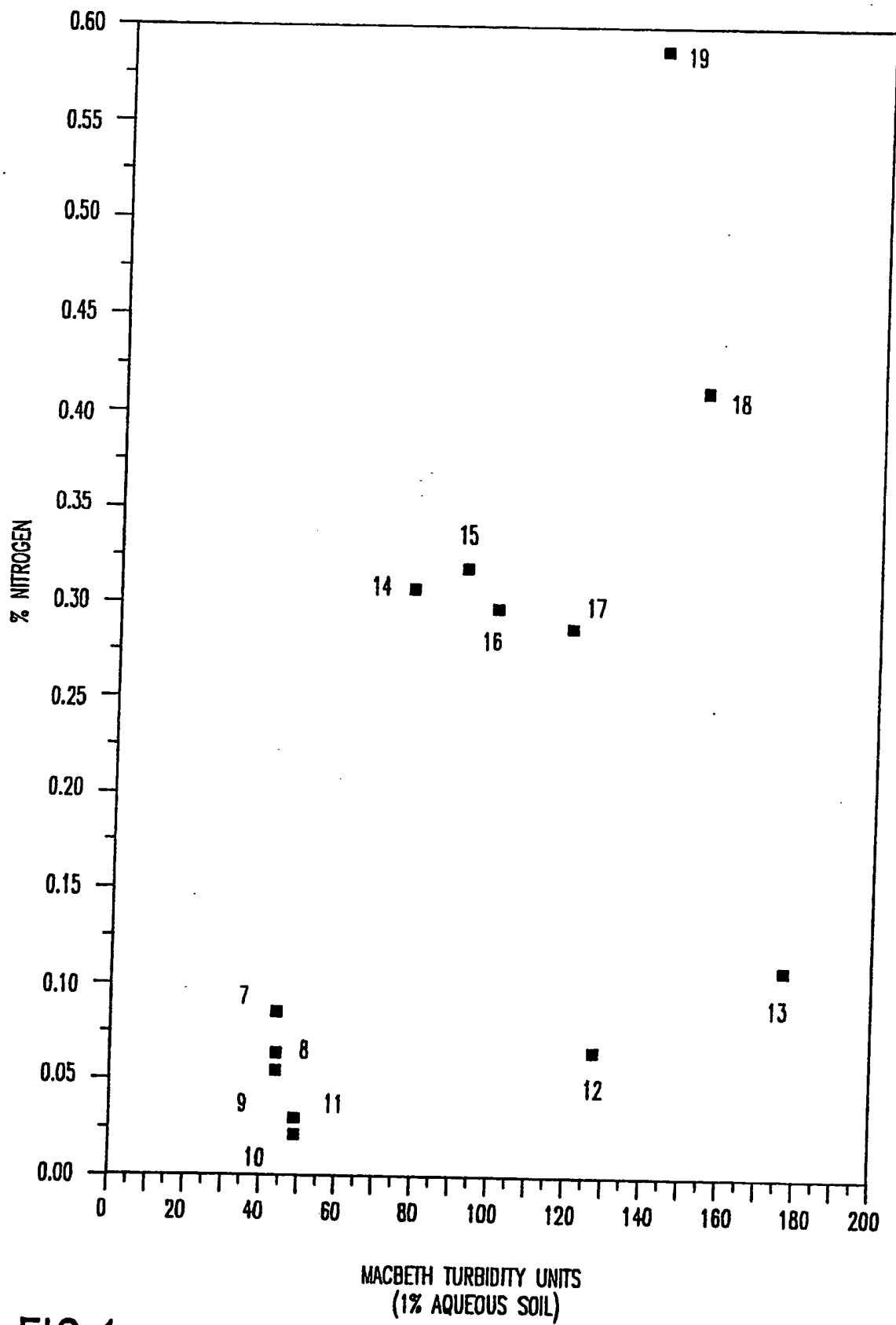


FIG.1

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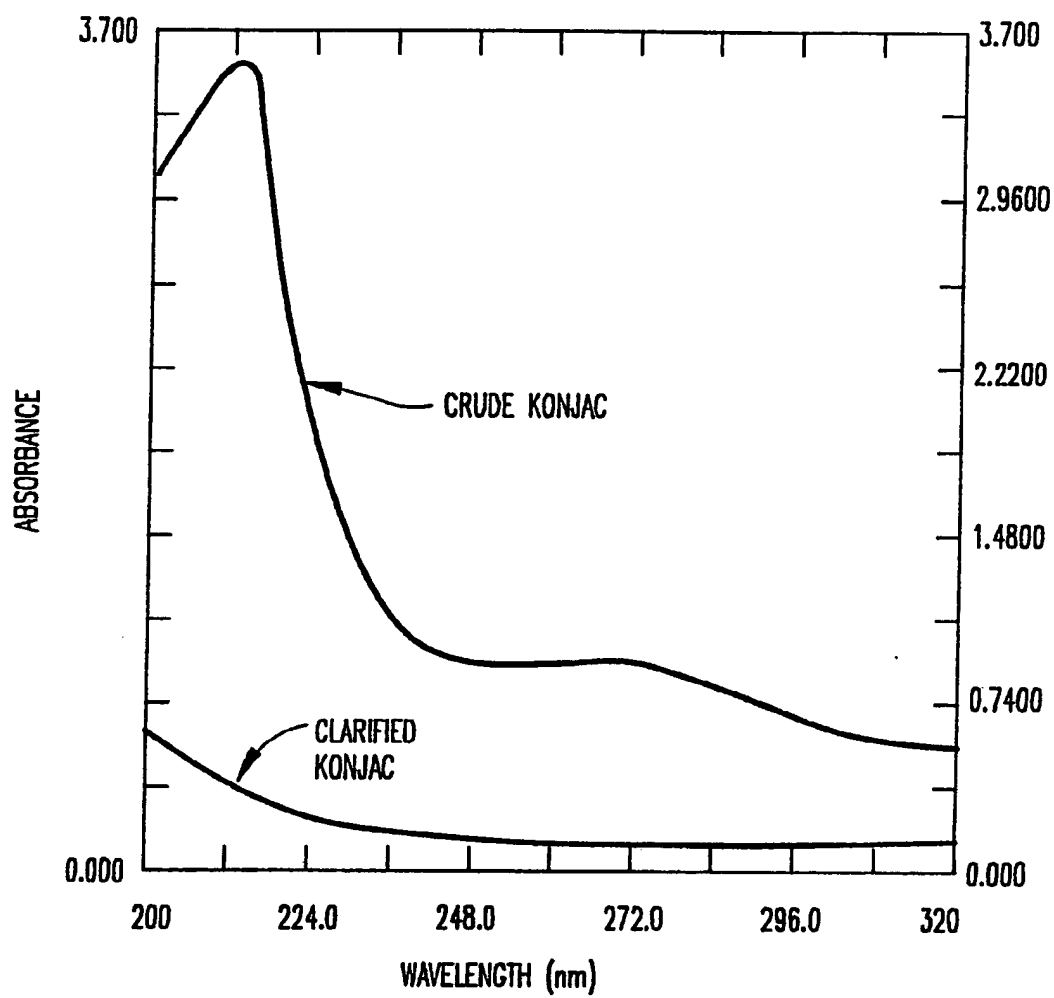


FIG.2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06591

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A23L 1/05, 1/20; C08B 37/00; C13K 1/00; C07K 1/08

US CL :252/315.3;426/573,46;536/114,123,127,128

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 252/315.3;426/573,46;536/114,123,127,128

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Documents cited in the prior U.S. application.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS TEXT; USPAT AND JPO; (KONJAK# OR KONJAC#) AND GEL #; NITROGEN; (CLEAR OR CLARIFIED)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP,A, H2-231044 (FUKUDA), 13 SEPTEMBER 1990, See entire document.	1-8, 15-16, 19-20, 22-26
Y	JP,A, 59-227267 (HARA), 20 DECEMBER 1984, See entire document (English translation).	17-18, 28-29 and 30-37
Y	US,A, 2,508,726 (RAMSTAD ET AL.), 23 MAY 1950, See entire document.	1-8, 19-26
Y	US,A, 4,746,528 (PREST ET AL.), 24 MAY 1988, See entire document and particularly columns 2 and 3.	9-14
Y	US,A, 4,427,704 (CHENEY ET AL.), 24 JUNE 1984, COL 1-4.	9-14
Y	Derwent Abstract, Accession Number 84-021155/04, (Corresponding to J58-213001-A), 10 DECEMBER 1983. 1-8 AND 19-26.	1-8 AND 19-26

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

28 OCTOBER 1992

Date of mailing of the international search report

25 NOV 1992

Name and mailing address of the ISA/
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06591

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,O	<u>Nutricol Konjac Flour</u> , (1989) FMC Corporation, Marine Colloids Division , Philidelphia, PA 19103, USA.	1-37
Y	US,A, 2,767,167 (OPIE ET AL.), 16 OCTOBER 1956, See col 1.	19-26
A	US,A, 2,599,771 (MOE), 10 JUNE 1952, See col 1 and claims.	19-20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/06591

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7 and 8
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. (Telephone Practice)
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/06591

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-6, 7-24 and 29-37 drawn to clarified Konjac glucomannan, a sol, a gel, and a method of preparing clarified Konjac glucomannan, classified in Class 536, subclass 114.

II. Claims 27-28 and 40-47, drawn to a cold melt gel, and methods of making a cold melt gel, classified in Class 252, subclass 315.3.

III. Claims 25-26 and 38-39, drawn to a spongy mass and method of making said spongy mass, classified in Class 426, subclass 573.

To inventions are distinct, each from the other because of the following reasons:

Inventions of Group I and Groups II or III are related as mutually exclusive species in intermediate-final product relationship. Distinctness is proven for claims in this relationship if the intermediate product is useful to make other than the final product and the species are patentably distinct.

In the instant case, the intermediate product is deemed to be useful as an additive in various forms and does not require the particulars of any one form. For example, the glucomannans may be used in the sol, gel, spongy network, powder as an absorbent, etc...